The Technology of Closed System Culture of Salmonids

Contents

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Introduction

Closed culture and waste removal

The advantages of employing water re-use systems in fish culture where natural waters do not meet the requirements for optimum production throughout the year are numerous, and most of these have been set forth by Burrows and Combs (1968). An additional advantage of considerable importance today is eliminating the discharge of nutrient-enriched waters.

The culturing of salmonids and other species of fish is attended by the production of waste metabolites that must be removed from the water if it is to be safely re-used. The large volumes of water involved and the relatively low metabolite levels pose special problems that we have solved using a multidiscipline approach. Our effort has been supported by microbiologists, a veterinary pathologist, engineers and fish culturists. Although our systems do not represent optimal designs, they have proven to be functional and capable of supporting salmon from incubation through grow-out to minimum market size.

This report will describe our smolt production and grow-out facilities as well as our solutions to typical operational problems—control of temperature and water flow rate, and oxygenation. It will also describe the typical problems and operations related to waste removal—clarification and the control of sulfide and nitrite toxicity. A major section will outline our approach to ammonia control and denitrification.

An economic evaluation of closed system salmon production is in progress, and it is anticipated that this project will assist in determining the economic feasibility of producing both smolt and market-sized fish. It is not the purpose of this report to discourage alternate production methods such as raceway culture, cage culture, net culture or sea ranching but rather to provide basic information on closed system culture that will assist in determining the method best suited to conditions existing at a particular location.

The facility: smolt production

The production of salmon smolts in our facility is a conventional operation, with the exception of water re-use made possible through the use of a physical filter and a nitrification filter.

Chinook salmon eggs were supplied by Dr. Lauren Donaldson of the University of Washington and by Mr. Richard Noble of the State of Washington Department of Fisheries. Incubation of eggs is carried out in eight-tray, "Heath Techna" incubators. Water supplied to the incubators

is taken from the smolt production re-use system, and a temperature of 10°C is maintained. Mortality experienced from the receipt of eyed eggs to swim-up fry has not exceeded five percent for two successive years.

Upon transferring sac fry from the incubator trays to spawn tanks the water temperature is increased to 13°C. Temperature is controlled indirectly through air temperature control in the culture building. The six spawn tanks used in the smolt production unit are commercially available and are designed for culturing Atlantic salmon fry.* Each tank measures 142 centimeters by 142 centimeters and is 45 centimeters deep. These tanks are bottom-discharging and are supplied with water from a 7.6-centimeter polyvinyl chloride (PVC) manifold that is gravity-fed from a pair of biological filters. Flow rates are adjustable but do not exceed 56 liters per minute. Water is discharged from the bottom through an external swing pipe to a collection sump and is then pumped over physical filters and into the aerobic filters. The physical filters have 40-mesh stainless steel screens which remove the bulk of the particulate matter from the culture water. The aerobic nitrification filters consist of polyethylene tanks 152 centimeters high and 132 centimeters in diameter. Each tank contains 1.132 cubic meters of plastic filter media. Aeration for the system is provided by two perforated pipes located beneath the plastic media and supplied with air from a source of 420 grams per square centimeter. This system contains 7500 liters of water and is capable of supporting 16,000 Chinook salmon fingerlings averaging 7.6 centimeters in length. Frequent feeding is done via programmed automatic feeders. All feeds used in the production of smolts are of commercial origin. These range in protein content from 50 percent for the starter to 38 percent for small crumbles used with larger fish.

Water quality is maintained by diluting it with fresh well water. Water re-use ranges from 90 to 95 percent. Ammonia levels are kept below 0.75 milligram per liter at pH 6.7 to 7.2 and the nitrate is not allowed to exceed 100 milligrams per liter. No effort was made to treat waste solids or water discharged from the system.

Mortality experienced has been almost all due to *Saprolegnia sp.* (freshwater fungus) which at times has exhibited a high degree of virulence. The source of this infectious agent has not been determined. It could enter with the water supplied to the system, since no form of sterilization is employed. Dust and feed also may serve as carriers.

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* *Manufactured in Denmark by L. M. Glasfiber and marketed in the United States by Astra Pharmaceutical.*

Treatment with therapeutic levels of antibiotics and other agents in water re-use systems poses a special problem in that levels which affect the pathogen have been assumed to be capable of reducing nitrification. As a result we are presently engaged in a project designed to determine the effects of common chemotherapeutic agents and antibiotics on nitrification. We regard reduced ability to treat fish in a water re-use system as a serious drawback.

The facility: grow-out

Our salmon grow-out facility which is located outdoors consists of four silo-like tanks constructed of glass-reinforced polyester resin (GRP), with two tanks measuring 152 centimeters in diameter and 305 centimeters in height and the other two, 152 centimeters in diameter and 366 centimeters in height. These tanks are mounted on ten-centimeter-thick insulated bases and have an outer covering of five centimeters of polyurethane foam which is sealed with resin-impregnated glass matting. An overflow collector ring made of GRP, ten centimeters high and 15.5 centimeters wide, is attached to a point five centimeters below the top of the tank. A wooden service platform surrounds the tanks.

Water is supplied to the tanks through ten-centimeter and four-centimeter PVC pipes that discharge at the center of the tanks at a point 15 centimeters above the bottom. Water discharged from the tanks overflows into the collector rings from which it is conveyed to the filters through ten-centimeter PVC pipes. The tanks can be operated individually or coupled in series. Water is continuously recirculated at a rate up to 378 liters per minute through the four-centimeter pipe and periodically at up to 1500 liters per minute through the ten-centimeter pipe.

This facility provides for two separate culture units, each consisting of two tanks and associated biological filters, pumps, refrigeration and heaters. Water temperature is maintained within $\pm$ 1° of 14°C with the help of a five-ton refrigeration unit during the summer months and direct steam injection heaters during the winter. Automatic controls are provided for both heating and cooling.

Each culture unit has a planned capacity of 1000 kilograms of 0.5-kilogram or larger salmon, although maximum capacity should approach 1500 kilograms. The total water volume in each system is approximately 18,500 liters, but may vary slightly according to the height of the water column in the nitrification filters.

Design of the nitrification filters used with this system represents a considerable departure from the more widely used gravel bed designs. Two filters support each system. These normally operate in parallel, but either one can be removed from service at will. Filter dimensions are 122 centimeters wide by 183 centimeters long by 162 centimeters high and they are constructed of 19-millimeter marine plywood and reinforced externally with five sets of five-centimeter by 15-centimeter oak reinforcing rings bolted at the corners. Two air dispersing lines measuring 2.5 centimeters in diameter run the length of the filter and are supported five centimeters off the bottom. The low-volume and high-volume water circulating lines are coupled to opposite ends of the filters through 3.8- and 7.6-centimeter bulkhead fittings, which are centered and 7.6 centimeters above the bottom. All water discharges from the filters are valved. Additional valved sampling ports are provided. The filters are packed with six preformed PVC modules having the following characteristics:

Material:	Rigid Polyvinyl Chloride
Color:	Gray
Volume:	16 ft^3 per module (0.529m^3)
Surface Area:	27 ft^2/ft^3 (88.6m^2/m^3)
Void Ratio:	97%
Weight:	2.4 lbs/ft^3 (384 g/10,000 cm^3)
Welding:	All contact surfaces solvent-welded
Flanging:	Top and bottom surfaces of modules

The relatively small surface area per unit volume provided by the filter elements is offset in part by the high void space (97 percent). This feature provides for a longer detention time which favors nitrification as illustrated in the following formula on the effect of detention time on nitrification developed by Haug and McCarty (1971):

$$t_o = [10^b/a(b-1)](1/S_e^{b-1} - 1/S_i^{b-1})(1+R) \quad (1)$$

where

t_o = detention time required to reduce the NH_3-N concentration from S_w to S_e, based on the raw waste flow and the filter void volume

S_i = influent concentration to the filter after mixing of waste and recycle flows

S_e = effluent concentration from the submerged filter

R = recycle ratio necessary to reduce concentration S_w to a level stoichiometric with the influent oxygen concentration
S_w = raw influent concentration before mixing with recycle flows
a = rate constant (0.11T-.20)
b = order of reaction (ave. 1.20)
T = temperature in degrees centrigrade

S_i can be calculated using the following formula:

$$S_i = (S_w + RS_e)/(1+R) \quad (2)$$

The detention time for a single-pass filter can be determined by modifying the above formula to:

$$t_o = [10^b/a(b-1)](1/S_e^{b-1} - 1/S_w^{b-1}) \quad (3)$$

Our system is operated as a flooded filter and can easily handle hydraulic loads of up to 35 liters per 1000 square centimeters per minute. The filter is subjected to continuous aeration in order to achieve internal circulation and to insure that the water leaving the filter is saturated with oxygen.

The system's configuration employed does not represent the ideal but is a compromise necessitated by the available space. Since water movement through a closed system can represent a significant part of the operating cost, it must be efficient and economical. In a water reuse system economies can be achieved by minimizing the distance that water must be lifted to move from one part of the system to the other. Quite obviously the layout of the nitrification filters in our system results in a greater lift requirement than would be required had they been designed for a 244-centimeter water column instead of a 145-centimeter column. A rectangular filter box rather than a cylindrical tank for housing the filter modules is also more expensive to build. Continuous water circulation for the system is provided by a one-horsepower open-impeller centrifugal pump. A two-horsepower conveyor pump is used to supply a high volume flow of 1500 liters per minute, used intermittently to flush the culture tanks.

Tertiary water treatment is employed on a continuous basis. Consisting of denitrification and clarification, it is carried out on a side-stream, taken from the bottom of one of the nitrification filters. The effluent from the denitrification column is returned to the second aerobic filter. (Operation and details of the denitrification treatment are covered in a subsequent chapter.)

Commercial salmonid grower feeds are used during grow-out operations. Experience is still limited, but it would appear that grow-out to minimum market size is possible in either fresh or brackish water (salinity of 12 to 15 parts per thousand). Chinook salmon smolts introduced in August of 1972 reached two pounds in weight by June 1973 with an average feed conversion of 1.15 kilograms of feed per kilogram of gain. Only one disease was encountered during this period; approximately 10 percent of the fish harvested exhibited kidney abnormalities that were diagnosed as kidney disease. A second study, involving smolts transferred to the silos in July 1973, is in progress. These fish averaged 250 grams in weight by February 1974, with exceptional individuals exceeding 400 grams.

Growth rate and feed conversion studies at a salinity of 12 parts per thousand will be initiated in the near future.

Some Basic Operations

Water flow rates

The siting of salmon and trout hatcheries has been to a considerable extent dictated by the availability of high quality water. Ideally operations should be carried out where there is a non-restricting supply of water meeting the optimum requirements for culturing a particular species. Unfortunately few locations meet these requirements and water re-use systems are being developed to increase the production capacity of available sources of water. Considerable research has been carried out in what are essentially flow-through systems to establish the water flow rates needed to support a given load of fish. The "Morris C. Croker Fish Energy Charts" (1973) represent a compilation of data developed in western salmonid hatcheries that can be used to determine water flow requirements under a wide range of conditions. According to the chart developed for Chinook salmon, a flow of 12 gallons per minute would be required to maintain 100 pounds of six-inch fish held at 57°F.

In terms of metric equivalents the required flow would be 100 liters per minute for 100 kilograms of 15.24-centimeter fish at 14°C. Thus the daily flow amounts to 144,000 liters per 100 kilograms. This volume provides 576 grams of oxygen if the level is reduced from ten milligrams per liter to six milligrams per liter and prevents the average ammonia level from exceeding 0.4 milligram per liter. Similar flow rates are employed in our smolt production system, and they are representative of systems employing aeration to achieve oxygen saturation of the culture water.

Flow rates in our silo culture system are quite different. In this system there are two different requirements; the first is based on the minimum vertical velocity necessary to remove particulate material, and the second, on the minimum flow necessary to remove dissolved metabolic waste products. Oxygen requirements are met by direct oxygenation (which is covered in a separate section). A high volume flow of 725 liters per minute will provide the vertical velocity (40 centimeters per minute) required, and in our system which delivers 1500 liters per minute, two silos can be flushed simultaneously when they are operated either in series or parallel. Flushing operations are automated and duration and frequency can be varied. The silos can be kept free of particulates when a high volume flow is used for 15 minutes every two hours.

It has been our experience that up to 1.5 milligrams of ammonia-nitrogen (NH_3-N) per liter can be tolerated over extended periods when the culture water is maintained in the range of pH 6.2 to 6.5.* With a

* *NH_3-N as used in this paper includes both NH_4^+ and NH_3-N.*

nitrification efficiency of 70 percent it is possible to provide a continuous supply of water to the silos at or below 0.45 milligram per liter. The minimum flow rate necessary to support 100 kilograms of 15.24-centimeter Chinook salmon at 14°C under these conditions is 39 liters per minute, or 33 percent of that recommended for a flow-through system. At maximum stocking density, a silo 1.52 meters in diameter by 3.66 meters high has a capacity of 400 kilograms of Chinook salmon averaging 15.24 centimeters in length. Using this as a basis, the average total flow required to maintain 100 kilograms would be 62 liters per minute.

Temperature control

One of the major advantages of having a water re-use system is the high degree of environmental control that can be exercised. This is particularly applicable to temperature which can readily be maintained in the optimum range, necessary to achieve maximum growth rates. It is not practical to do this in the majority of state and federal hatcheries, and as a result the time required to raise fish to release size in such places is extended. In the case of Atlantic salmon hatcheries up to three years are required to produce a smolt.

For purpose of illustration let us assume that it is desirable to raise the temperature of available water from 8°C to 14°C in a flow-through system supporting 100 kilograms of 15.24-centimeter Chinook salmon. With a flow of 100 liters per minute the energy required can be calculated using the following formula:

$$V_T \times W \times 1440 \times dT \times Spht = \text{energy required/day} \quad (4)$$

where

V_T = volume flow/minute
W = weight/unit volume
dT = temperature difference
$Spht$ = specific heat of water

Substituting the given values into this formula we have:

$$100 \times 1 \times 1440 \times 6 \times 1 = 864{,}000 \text{ kilogram calories} \quad (5)$$

This high energy requirement and its cost make thermal manipulation uneconomical in most flow-through systems. In water re-use systems the energy requirement for maintaining the desired temperature is

greatly reduced because of the high re-use capability. But a number of factors determine the energy required to maintain the desired temperature: the difference between the air temperature and water temperature, the amount of water surface exposed to the air, the rate of air movement over the surface, relative humidity of the air, the amount and kind of insulation, temperature of makeup water and the percent of re-use being employed. Unless all these factors are considered, total energy requirements cannot be determined. We have not established all of these factors for our system but the experience of operating with an air temperature of −18°C indicates that steam requirements are very low for maintaining a water temperature of 14°C with 95 percent re-use.

When high air temperatures are encountered it may be necessary to employ refrigeration if water temperatures cannot be maintained by reducing re-use and increasing the percentage of flow through. Under conditions in our system where summer air temperatures may exceed 35°C, we have successfully employed refrigeration to maintain the desired culture temperature. The amount of refrigeration required depends upon the same factors needed to determine energy requirements for heating. Each of our silo systems contains 18,500 liters of water, and refrigeration is provided with a five-ton unit. During periods when the average air temperature is above 14°C a part of the low-volume continuous flow of water from the aerobic nitrification filters is diverted to the chiller barrel (evaporator) of the refrigeration unit. The percentage flow through the barrel can be adjusted by valves in the lines. With a mean ambient air temperature of 28°C the unit operates about 35 percent of the time.

Refrigeration provides for considerable flexibility in a water re-use system. In addition to holding temperature at the optimum level for growth, it can be used to provide sub-optimal temperatures, thus enabling control of the growth rate. This feature is particularly desirable for a commercial salmonid production facility, needing a supply of smolts for stocking grow-out facilities on a year-round basis from a single hatching.

Oxygenation

The quantity of fish that can be carried in a system is closely related to that system's ability to provide the oxygen needed. Where intensive culture is practiced, flow rates are a function of oxygen demand. Such factors as temperature and salinity affect the oxygen-carrying capacity

of water; for example, increases in temperature or salinity result in a decrease in oxygen. For most salmonids the oxygen content should be maintained at six milligrams per liter or higher. Under some conditions salmonids can adapt to lower concentrations, but best results can be expected when the oxygen level is near saturation, typical of the natural environment.

In order to reduce water flows to a minimum we have resorted to direct oxygenation in our silo system. This choice has the added advantages of insuring an adequate level of oxygen in the event of a power or mechanical failure and/or increasing immunity to ammonia. The full extent of the latter advantage has not yet been developed.

Oxygen requirements can be approximated using Liao's (1971) equation:

$$O_2 = K \cdot T^n \cdot W^m \qquad (6)$$

where

O_2 = oxygen uptake rate in lbs O_2/100 lbs fish/day
K = rate constant
T = water temperature in degrees Fahrenheit
W = fish size lb/fish
m,n = slopes

For different species at different water temperatures, the values of K, m and n have been established by Liao (1971) and are tabulated below:

Species	Temperature	K	m	n
Salmon	$T \leq 50°F$	7.2×10^{-5}	−0.194	3.200
	$T > 50°F$	4.9×10^{-5}	−0.194	2.120
Trout	$T \leq 50°F$	1.9×10^{-6}	−0.138	3.130
	$T > 50°F$	3.05×10^{-4}	−0.138	1.855

Using the above formula, the oxygen requirements for 100 kilograms of 15.24-centimeter Chinook salmon at 14°C can be approximated:

$$O_2 = 4.9 \times 10^{-5} \times (57.2)^{2.12} \times (0.10)^{-0.194} = 0.4072 \text{ lbs/100 lbs fish/day} \qquad (7)$$

$$O_2 = 0.4072 \times 454 \times 2.2 = 407 \text{ g/100 kg fish/day} \qquad (8)$$

Liquid oxygen is used as the primary oxygen source in our silo culture system. This product is readily available and can be stored in cylinders designed for the pressures encountered. As long as the demand is established a container of appropriate size can be used with little loss

due to venting to the atmosphere to reduce pressure. At temperatures normally encountered sufficient gas is generated in the container to supply the system. Oxygen is supplied to the culture water in the silos by introducing gaseous oxygen through diffusers. A stainless steel diffuser is located on the bottom and at the center of each silo. The incoming water is discharged directly above the diffuser plate, a technique which facilitates dispersion of the oxygen. With a water column exerting a pressure of 304 grams per square centimeter supersaturation is readily achieved and oxygen absorption is complete. Oxygen concentrations of up to 18 milligrams per liter at the bottom of the silos have been used without apparent adverse effects. Addition rates are determined by measuring the concentration at the top of the silo. The discharge concentration is maintained at six milligrams per liter or more. Flow meters will be installed in all oxygen lines in the near future to enable us to make a more accurate determination of the amount consumed.

Direct oxygenation has been employed for 12 months with excellent results. Numerous power and mechanical failures during this period have occurred without loss of fish.

Clarification

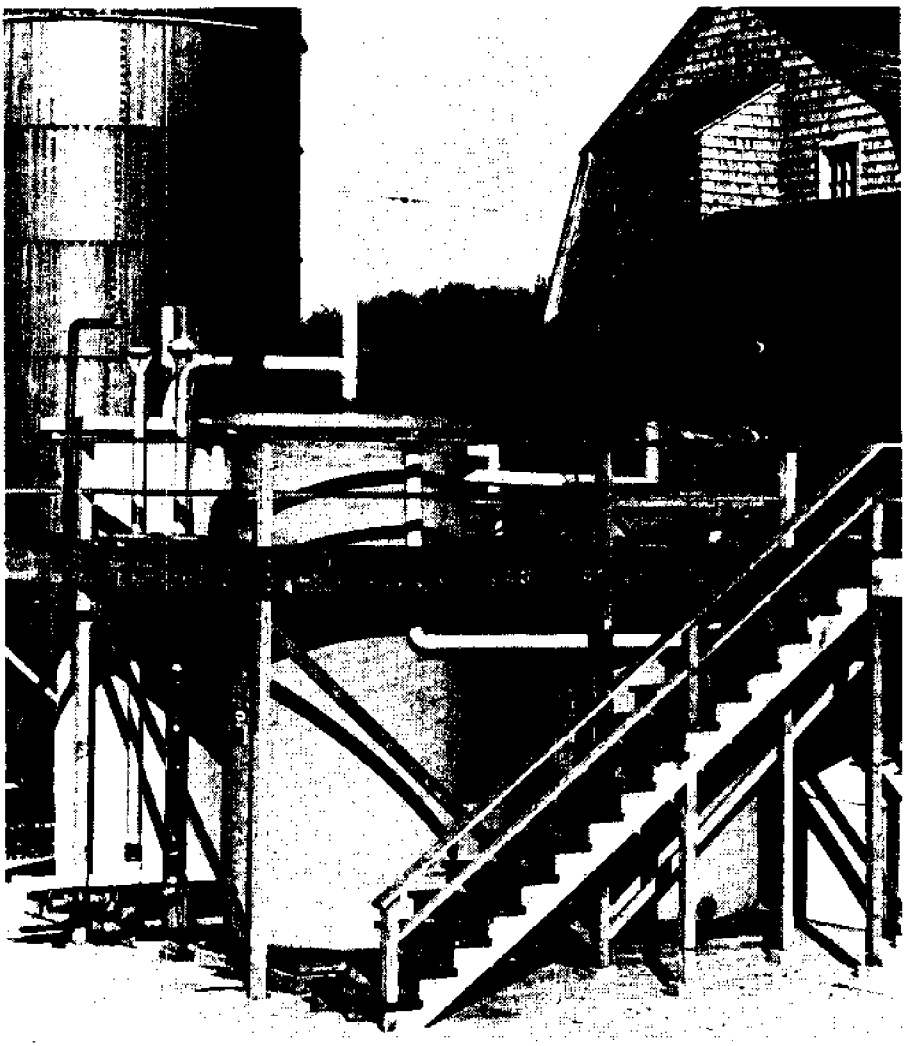
Removal of particulates from culture water is accomplished in two steps. All water coming from the culture systems is passed over a 40-mesh stainless steel screen mounted above the aerobic filters. The bulk of the larger particulate material is effectively screened out by this operation. There is a tendency for the screen to foul as a result of bacterial growth; when this happens the contents of the box holding the screen overflows into the filter. The effects of fouling can be minimized by frequent brushing and washing. Steam cleaning is more effective because it removes slime and kills the bacteria responsible for its formation.

Finely divided particulate material, which has a tendency to build up, is removed by sedimentation. A clarifier tank is installed between the denitrification filter and the nitrification filter. All water being returned to the system from the denitrification column passes through the clarifier before entering the aerobic filter. With a detention time of 50 to 80 minutes, the particulates settle out and can be removed from the bottom of the clarifier.

The underflow which contains particulate from the clarifier is discharged from the system to a sewage disposal plant. We have made no

attempt to treat the solid waste from our operation, but recognize the necessity for dealing with this problem in the future.

We do not regard our approach to clarification as entirely satisfactory and wish to direct attention to the importance of this operation. Anyone contemplating pilot- or larger-scale operations should review established methods for water clarification and select the most appropriate for cost effectiveness evaluation.



Ammonia Control and Denitrification

Calculation of ammonia production

Ammonia production is closely related to the feeding rate (R_F), dietary protein-nitrogen level (N_L), protein utilization (N_U) and nitrogen excreted as ammonia or compounds readily converted to ammonia (N_E). This relationship is shown in the following formula:

$$R_F \times \text{Biomass} \times N_L \times N_U \times N_E = NH_3\text{-N production} \quad (9)$$

The following example shows a method for calculating ammonia production for a 24-hour period. Given a fish biomass of 100 kilograms, feeding rate two percent of body weight, protein level in diet of 34 percent (5.44 percent nitrogen) protein utilization of 40 percent and 90 percent of nitrogen excreted as ammonia:

$$.02 \times 100 \text{ kg} \times .0544 \times .6 \times .9 = .0587 \text{ kg of } NH_3\text{-N/day} \quad (10)$$

The above formula is simplified, is based on nitrogen balance and does not differentiate between exogenous and endogenous nitrogen. It should be recognized that nitrogen would be excreted by the fish if the feeding rate were reduced to zero. The efficiency of protein utilization is the variable having the most significant effect on ammonia production. Properly balanced diets, particularly with respect to protein and energy, will help to maximize protein utilization. Thus, optimal diets are not only more economical in terms of protein, but they also minimize the amount of nitrogen excreted.

Ammonia is a toxic metabolite and if allowed to accumulate in a culture system will cause high mortality. Generally, as mentioned previously, the volume or flow of water in a culture system is dictated by the need to provide oxygen to remove waste products including ammonia.

Control of nitrite toxicity

Nitrite is not a primary product of fish metabolism and therefore does not pose a problem in flow-through systems, but it is a product of the microbiological oxidation of ammonia and is present in culture water in re-use systems. Westin (1973) has established the 96-hour LD_{50} of nitrite for Chinook salmon at 2.5 milligrams per liter in fresh water. Liao (1972) has reported that when fish were exposed to a nitrite level as low as 0.15 milligram per liter of nitrite-nitrogen (NO_2-N) for 48 hours, about 72 percent of the hemoglobin in the blood was converted to

methemoglobin. Considerable mortality was experienced at NO_2-N concentrations between 0.2 and 0.3 milligram per liter.

We have observed NO_2-N levels in our water re-use silo system ranging from 0.08 to 0.20 milligram per liter when nitrification was the only water treatment employed. Although we did not find mortality attributable to the level of nitrite present we did notice a lack of stamina in the fish. Denitrification provides the means for reducing NO_2-N levels to safe concentrations; effluent water from our denitrification column has consistently shown NO_2-N levels below 0.02 milligram per liter.

In water re-use systems where denitrification is not employed there is the ever present danger of chronic nitrite toxicity and it is recommended that the nitrite levels be monitored and, if excessive mortality is encountered, that methemoglobin levels be determined.

Control of sulfide toxicity

Hydrogen sulfide like nitrite seldom constitutes a problem in flow-through systems with a good water supply. Since it is produced under anaerobic conditions, it is not normally encountered in water re-use systems employing nitrification unless a part of the filter becomes clogged and the flow of water is diverted around the area. This can occur in fine-media filters which are not adequately backflushed. Filters using plastic media with a high percentage of void space are not prone to clogging and are unlikely to become anaerobic.

When denitrification is employed, the possibility of producing hydrogen sulfide is increased since the reactions are carried out at greatly reduced oxygen levels (0.5 milligram per liter). Sulfate reduction can be avoided by carefully controlling the dissolved organic carbon level in the influent stream. Addition of supplemental carbon in excess of that required for denitrification will result in the production of hydrogen sulfide. This occurred in the initial activation of our denitrification column. Effluent water from the column was re-aerated and bio-assayed to determine safety. Total mortality was experienced within 12 hours. Unfortunately, sulfide levels were not determined; however, the characteristic odor was detectable. Once the problem was recognized, it was corrected by reducing the level of organic carbon.

If the nitrate level in the influent to a denitrification column is variable, we recommend that the total organic carbon level be adjusted to the lowest nitrate concentration rather than to the highest. When the

level of organic carbon is less than that required for denitrification the percent of nitrate reduced is lowered but the nitrite reduction is not lowered so there is no apparent danger of discharging water high in nitrite.

Hydrogen sulfide is extremely toxic to fish and also inhibits nitrifying bacteria, so its production should be avoided.

Nitrification

The accumulation of waste products in a fish culture system restricts the re-use of water. Under some conditions, water may be reused from three to four times without detrimental effect; however, in a high density culture system (where densities may reach 100 grams per liter) this cannot be done without resorting to very high flow rates. Feeding salmonids (100 grams) at two percent of body weight at this density will result in an average production of 2.45 milligrams of NH_3-N per hour, thus necessitating a minimum of three volume changes per hour to maintain the ammonia at an acceptable level.

Thus, to achieve high water re-use capability it is necessary to either remove ammonia or convert it to a less toxic compound. This can be done by chemical, physical and biological methods (to be discussed later in this chapter). Most of our experience centers on the use of microbiological oxidation of ammonia to nitrate, a less toxic nitrogen compound. Although this process is widely used in the treatment of sewage and industrial wastes it is not particularly efficient when applied to salmonid culture water. This is largely due to temperature (14°C) and the low nutrient concentration (average one milligram NH_3-N per liter).

The effect of temperature and NH_3-N concentration on the rate of nitrification has been set forth by Haug and McCarty (1971) who developed the following average rate equation for the temperature range 5 to 25°C:

$$-ds/dt = (0.11T - 0.20)(s/10)^{1.2} \qquad (11)$$

where:

$-ds/dt$ = the rate of NH_3-N oxidation at any point in a submerged filter in mg/l − min
s = NH_3-N concentration in mg/l
T = temperature in °C

Using the above formula, an increase in temperature from 15 to 25°C, with an NH_3-N concentration of one milligram per liter results in a 1.76-fold increase in reaction rate. Raising the NH_3-N concentration from one milligram per liter to ten milligrams per liter, at 15°C, will result in a 15.86-fold increase in the reaction rate. Thus, the NH_3-N concentration to a large extent controls the reaction rate.

Gigger and Speece (1970), working with rainbow trout held in 21°C water, obtained nitrification rates up to 62.5 milligrams of NH_3-N per 1000 square centimeters per day in a submerged, forced-circulation gravel filter, packed with stones averaging 1.9 centimeters in diameter. In a Florida catfish operation (1973) where water temperature was maintained at 30°C and the NH_3-N concentration allowed to rise to 2.05 milligrams per liter, nitrification rates up to 81 milligrams of NH_3-N per 1000 square centimeters per day were obtained using a plastic media trickle filter. The effect of nutrient concentration on the oxidation rate has been demonstrated by Duddles and Richardson (1973) who obtained rates up to 168 milligrams per 1000 square centimeters per day using a plastic media trickle filter with an influent stream at 30°C and a concentration of 14.6 milligrams of NH_3-N per liter.

From the foregoing, it is apparent that either trickle or submerged aerobic biological nitrification filters can be employed. As discussed previously, our systems use submerged filters and provide for high hydraulic loading rates. The use of PVC modules as support media represents a compromise between the surface area provided per unit volume and the void space which enables continuous operation without backflushing. The PVC modules are commercially available. Measuring 60.96 centimeters by 60.96 centimeters by 121.92 centimeters, they provide 40.13 square meters of surface area for bacterial growth. Nitrification rates of up to 75 milligrams of NH_3-N per 1000 square centimeters per day have been achieved in a partially loaded system of this type. Higher rates have been obtained using an aerated test module by increasing the NH_3-N concentration with the addition of ammonium chloride. With temperature limited to 14°C in our culture systems higher nitrification rates will depend upon an increase in the NH_3-N influent concentration. Sousa *et al.* (1974) have recently reported that ammonia immunity can be increased by environmental manipulation; however, until these data are confirmed on a pilot-sized operation, we would recommend that our more conservative observed rates be used for design purposes.

On the basis of the nitrification rates obtained in our systems, one can calculate the filter surface area required for a given loading and

feeding rate. (See calculation of ammonia production rates in the first part of this chapter.) Using the value of 58.7 grams of NH_3-N per 100 kilograms of fish, one can calculate the filter surface area required by use of the following simplified formula:

$$F_A = N_p/N_o \qquad (12)$$

where:

F_A = surface area required in m^2
N_p = g NH_3-N/unit of biomass/day
N_o = g NH_3-N oxidized/m^2/day

Substituting ammonia production and oxidation rates in this formula, we have

$$F_A = 58.7/.75 = 78.26 \text{ m}^2$$

Thus, two PVC modules would be required to support the bacterial population necessary to bring about the oxidation of ammonia produced by 100 kilograms of fish under the conditions indicated.

Although PVC modules have been successfully employed in our systems development work, they do not necessarily represent the most ideal filter media. Under some conditions alternate forms of plastic media or natural materials, such as sand, gravel, stone or coal, may be more desirable. A comparison of the surface areas provided by available media is presented in the following table.

Surface areas of nitrification filter media.

	Media			
	No. 8 Stone	*1.9 cm Stone*	*2.54 cm Flexirings*	*PVC Modules*
Specific surface area m^2/m^3	584	279	195	88.6

Where low flow rates are employed and backflushing of the filter is not objectionable stone media have advantages in cost and in surface area provided per unit volume. Small-diameter polypropylene flexirings also provide more surface area than the PVC modules employed in our system.

Chemistry and microbiology of nitrification

It is the high order of solubility of ammonia in water that restricts the methods which can be used for its removal. Synthetic and natural

zeolites have been used, but bacterial nitrification appears to offer the most practical and economic method for its removal. This is particularly so in marine systems where dissolved salts interfere with absorption of the ammonium ion.

Biological nitrification is carried out by two general groups of aerobic autotrophic bacteria, *Nitrosomonas*, which oxidizes ammonia-nitrogen (NH_3-N) to nitrite-nitrogen (NO_2-N), and *Nitrobacter*, which oxidizes nitrite-nitrogen to nitrate-nitrogen (NO_3-N) as their energy sources. These organisms require an oxygen-rich environment as their energy source and inorganic carbon in the form of carbon dioxide or bicarbonate as their carbon source.

The yield of energy from the oxidation of ammonia to nitrite to nitrate is relatively small. Thus, a considerable quantity of these compounds must be oxidized to yield cell growth. It has been proposed (Anon. 1971) that the synthesis-oxidation equations are as follows:

Nitrosomonas
$$55\ NH_4^+ + 5\ CO_2 + 76\ O_2 \rightarrow C_5H_7O_2N + 54\ NO_2^- + 52\ H_2O + 109\ H^+ \quad (13)$$

Nitrobacter
$$400\ NO_2^- + 5\ CO_2 + NH_4^+ + 195\ O_2 + 2H_2O \rightarrow C_5H_7O_2N + 400\ NO_3^- \quad (14)$$

On the basis of these reactions, it is calculated that the cell yield of *Nitrosomonas* is 147 grams per kilogram of NH_3-N oxidized and the cell yield of *Nitrobacter* is 20 grams per kilogram of NO_2-N oxidized.

The high oxygen requirement for nitrification is shown by the following equations:

Nitrosomonas
$$2\ NH_4^+ + 3O_2 \rightarrow 2\ NO_2^- + 2\ H_2O + 4H^+ \quad (15)$$

Nitrobacter
$$2\ NO_2^- + O_2 \rightarrow 2\ NO_3^- \quad (16)$$

On the basis of these equations, Stankewich (1972) calculated the stoichiometric amount of oxygen required for the oxidation of NH_3-N to NO_2-N as 3.43 kilograms of oxygen per kilogram of NH_3-N oxidized. The amount of oxygen required for the oxidation of NO_2-N to NO_3-N is 1.14 kilograms of oxygen per kilogram of NO_2-N oxidized. This results in an oxygen demand of 4.57 kilograms of oxygen for the complete oxidation of a kilogram of NH_3-N.

The cellular synthesis equation for autotrophs proposed by Haug and McCarty (1971) is as follows:

$$NH_4^+ + 5\ CO_2 + 2\ H_2O \rightarrow C_5H_7O_2N + 5\ O_2 + H^+ \quad (17)$$

In the process of synthesis part of the oxygen requirement is obtained from the carbon dioxide used as a carbon source.

Haug and McCarty (1971) have proposed the following overall equation for the synthesis and metabolism of nitrifying organisms based on their calculated yield coefficients:

Nitrosomonas

$$29\ NH_4^+ + 37\ O_2 + 5\ CO_2 \rightarrow C_5H_7O_2N^+ + 28\ NO_2^- + 57\ H^+ + 26\ H_2O \quad (18)$$

Nitrobacter

$$96\ NO_2^- + 43\ O_2 + 5\ CO_2 + NH_4^+ + 2\ H_2O \rightarrow C_5H_7O_2N + 96\ NO_3^- + H^+ \quad (19)$$

These equations yield oxygen consumption ratios of 3.02 kilograms of oxygen per kilogram of NH_3-N oxidized to NO_2-N and 1.02 kilograms of oxygen per kilogram of NO_2-N oxidized to NO_3-N, or an overall oxygen requirement of 4.04 kilograms. Since this ratio will vary somewhat with the age of the culture, between 4.0 and 4.6 kilograms of oxygen will be required for each kilogram of NH_3-N completely oxidized.

The microbial population of a nitrifying filter is extremely diverse and includes both autotrophic and heterotrophic species. New systems that are lightly stocked with fish generally require considerable time to become "conditioned." Initially the heterotrophs, because of their comparatively short generation time, will quickly achieve dominance and consume most of the ammonia produced by the fish. But the lack of organic carbon normally limits their growth, and, thus, the early disappearance of ammonia or low ammonia levels in a system does not necessarily indicate nitrification. The establishment of an autotrophic population can be detected by observing the buildup of NO_2^--N and NO_3^--N. In newly activated systems there is a tendency for the NO_2^--N levels to rise before the NO_3^--N, a condition that can lead to problems since NO_2^- is very toxic to fish.

Filter pre-activation

In our work we find it advantageous to pre-activate nitrifying filters before introducing fish to the culture system. The procedure is relatively simple and consists of providing a synthetic culture medium and inoculating it with a source of *Nitrosomonas* and *Nitrobacter* species. These organisms are widespread in nature; thus, ordinary garden soil or

stream sediment can be used as a source of inoculum. It is also possible to use pure cultures which might be more desirable under particular conditions, when there is a danger of introducing specific pathogens and parasites, for instance.

In our filter-activation studies, nutrients shown in the following table were added to chlorine-free tap water to support the growth of nitrifying bacteria.

Synthetic media for growing nitrifying bacteria.

Tap Water	1000 liters
Dibasic ammonium phosphate [$(NH_4)_2HPO_4$]	40 grams
Dibasic sodium phosphate (Na_2HPO_4)	40 grams
Instant Ocean, solid form*	40 grams
Instant Ocean, liquid form*	0.5 gram
Calcium carbonate ($CaCO_3$)	250 grams

* *Supplied by Aquarium Systems, Inc.*

Dibasic ammonium phosphate was added as needed to maintain the desired level of NH_4^+-N, and a ten-percent solution of sodium bicarbonate was added as needed to maintain alkalinity and a pH of 7.5. Instant Ocean, a synthetic sea salt, was used as a source of sodium chloride and trace elements. This medium was used to develop a population of nitrifying bacteria in a number of filters employing gravel and plastic support media.

The efficiency of ammonia oxidation is largely a function of concentration, temperature, pH and detention time in a flooded filter. During activation optimum conditions can be maintained and a nitrifying capacity equivalent to that to be imposed by the fish load can be established in a relatively short period. Pre-activating filters has the added advantage of insuring that the microbial population will be essentially autotrophic in nature due to the extremely small amount of organic carbon available from the nutrient media. (We have maintained ammonia concentrations between ten and 20 milligrams per liter.) The temperature of the culture medium is increased at a rate of approximately 1.5°C per day from the time of inoculation up to 22°C. Higher temperatures than 22°C have not been resorted to since the ultimate temperature of the culture system is 13 to 14°C. Detention time does not affect filter pre-activation since the filter is normally isolated from the culture system and internal circulation and aeration are provided to insure adequate oxygen levels.

Denitrification

In a water re-use system employing microbiological nitrification to convert ammonia to nitrate, there is a progressive buildup of nitrate which may ultimately reach toxic levels. The LD_{50} of nitrate for Chinook salmon has been established as 5000 milligrams per liter in fresh water and 4000 milligrams per liter in salt water by Westin (1973). The levels that will interfere with normal growth are rather uncertain; we have not allowed the level in our system to exceed 200 milligrams per liter and normally operate at levels below 100 milligrams per liter.

Discharge of water high in nitrate would represent a significant source of pollution. In order to avoid this we have employed columnar denitrification with effluent water either returned to the system or discharged. Laboratory and pilot studies have established the feasibility of continuous microbiological denitrification, which results in the actual removal of nitrogen from the system in gaseous form.

In many respects denitrification is a more efficient phase of culture water treatment than nitrification. This is largely due to the fact that the influent NO_3-N level can be controlled. In our work NO_3-N levels up to 20 milligrams per liter have been present in the column influent. This is in marked contrast to the one milligram per liter of NH_3-N in the influent to the nitrification filters.

Three categories of nitrate reduction have been defined by Verhoeven (1956): (1.) assimilating nitrate reduction where nitrate is a source of nitrogen for synthesis under either aerobic or anaerobic conditions; (2.) dissimilatory nitrate reduction where the nitrate serves as the essential hydrogen acceptor under anaerobic conditions, and (3.) incidental nitrate reduction when the nitrate is a non-essential hydrogen acceptor. It can be accomplished by a variety of common facultative bacteria including species of the genera *Pseudomonas* and *Bacillus*. The nutrients required by these microorganisms can be supplied by the effluent from a nitrification filter. Usually such an influent, particularly in the case of a salmon culture system, lacks an adequate level of organic carbon to support the energy requirements needed to bring about the desired degree of nitrate reduction. But a number of common organic carbon compounds, such as glucose, molasses, ethanol and methanol can be used as energy sources, and methanol has been most widely used for this purpose.

The quantity of methanol needed for denitrification was determined by St. Amant and McCarty (1969) on the basis of its reaction with

nitrate as well as its ability to meet requirements for bacterial synthesis. The following equations illustrate the two-step process of denitrification:

First Step

$$NO_3^- + 1/3\ CH_3OH \rightarrow NO_2^- + 1/3\ CO_2 + 2/3\ H_2O \quad (20)$$

Second Step

$$NO_2^- + 1/2\ CH_3OH \rightarrow 1/2\ N_2 + CO_2 + 1/2\ H_2O + OH^- \quad (21)$$

Overall

$$NO_3^- + 5/6\ CH_3OH \rightarrow 1/2\ N_2 + 5/6\ CO_2 + 7/6\ H_2O + OH^- \quad (22)$$

From the overall reaction at least five to six moles of methanol are required for every mole of nitrate for the reaction to be complete; 1.9 milligrams per liter of methanol are needed for each milligram per liter of nitrate-nitrogen. If less than the required amount of methanol is added the reaction will not go to completion.

In his review of the literature Schroeder (1967) cites evidence of oxygen inhibition of denitrification. Oxygen removal can be accomplished biologically by the addition of more methanol:

$$O_2 + 2/3\ CH_2OH \rightarrow 2/3\ CO_2 + 4/3\ H_2O \quad (23)$$

In addition to the requirements for denitrification and de-oxygenation, methanol must be supplied to satisfy the requirements for bacterial growth. St. Amant and McCarty (1969) determined an additional 30 percent must be provided to satisfy this requirement. Based on these considerations, the following formula has been developed for determining the total methanol requirement for complete denitrification:

$$Cm = 2.47\ N_0 + 1.53\ N_1 + 0.87\ D_0 \quad (24)$$

where:

Cm = required methanol concentration in mg/l
N_0 = initial nitrate-nitrogen concentration in mg/l
N_1 = initial nitrite-nitrogen concentration in mg/l
D_0 = initial dissolved oxygen concentration in mg/l

An extensive study on columnar denitrification by Smith *et al.* (1972) further confirms earlier studies by St. Amant and McCarty (1969) and establishes the economic feasibility of this method of nitrogen removal. The finding of Davies (1973) that the facultative microorganisms responsible for nitrate reduction under anaerobic conditions are capable of utilizing methane as a source of energy should further improve the economics of this step in culture water treatment.

The column used in our system is made of glass-reinforced polyester resin and is 3.65 millimeters high and 0.61 millimeter in diameter. It is packed with 0.906 cubic meter of 2.54-centimeter polypropylene flexirings. Culture water from an aerobic filter is pumped in at the bottom of the column and the effluent is discharged back into one of the two aerobic filters in the culture system or to a drainage sump. A detailed description of the activation and operation of the column has been presented by Meade and Kenworthy (1974).

Influent and effluent nitrate and nitrate levels encountered in columnar denitrification.

Day	Influent (mg/l)		Effluent (mg/l)		% Reduction	
	NO_3	NO_2	NO_3	NO_2	NO_3	NO_2
12	68.20	0.33	7.44	0.13	89.1	60.6
13	68.20	0.23	7.44	0.06	89.1	73.9
14	55.80	0.41	10.54	0.07	81.1	82.9
15	57.04	0.39	9.30	0.10	83.7	74.4
16	58.28	0.45	8.68	0.07	85.1	84.4

It will be noted from the table that NO_3-N reduction ranges between 80 and 90 percent and NO_2-N between 60 and 85 percent. At no time have we encountered effluent NO_2-N levels above those in the influent. This is also true when the total organic carbon level is below the stoichiometric amount calculated for complete reduction. Control of methanol input in our system is maintained by a vari-speed-drive-powered, low volume, gear pump. The methanol addition rate is calculated according to the formula of St. Amant and McCarty (1969):

$$Cm = 2.47\ N_0 + 1.53\ N_1 + 0.87\ D_0 \qquad (25)$$

where

Cm = required methanol concentration in mg/l
N_0 = initial NO_3-N concentration in mg/l
N_1 = initial NO_2-N concentration in mg/l
D_0 = initial dissolved oxygen concentration in mg/l

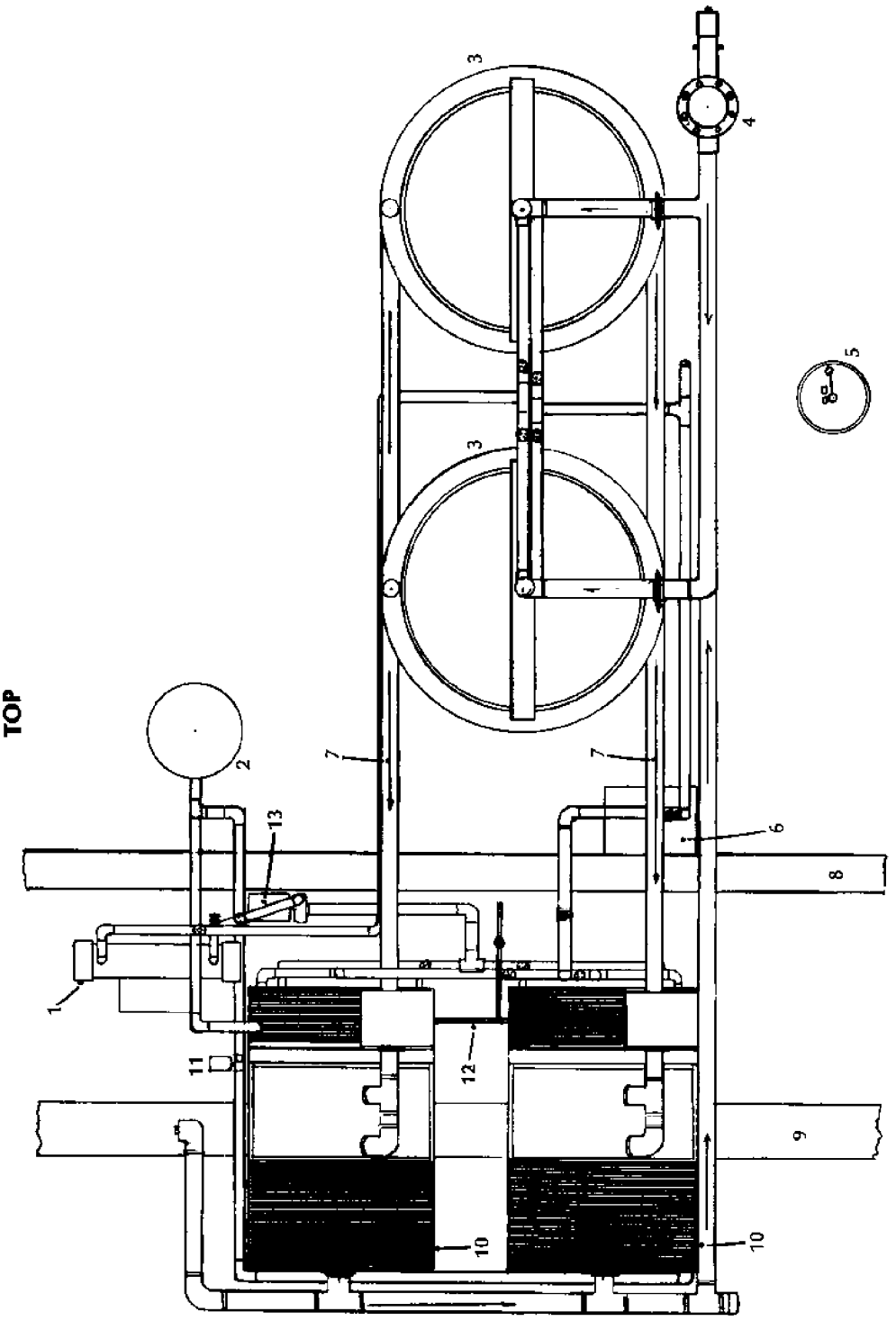
Flow rates four to 15 liters per minute have been employed, which in turn provide for detention times of 4.45 and 1.18 hours, respectively. Denitrification rates of up to 452.4 grams per cubic meter per day or 232 milligrams per 1000 square centimeters per day of NO_3-N have been obtained at an operating temperature of 14°C. This rate of denitrification is considerably lower than that reported by Smith *et al.* (1972) who

obtained a rate of 356 milligrams of NO_3-N per 1000 square centimeters per day on a filter packed with rock averaging 1.9 centimeters in diameter and operating at 27°C. This difference could be due to operating temperature and/or amounts of microbial biomass adhering to the surface of the support media.

As in the case of nitrification, alternate filter media can be employed, but we feel that the additional cost and smaller specific surface area provided per unit volume of plastic filter media is offset by the lower weight, ease of handling and high void space that eliminates the need for backflushing. Although our data are still preliminary, we feel that the denitrification rates achieved can be obtained or exceeded with scaled-up units.

If we continue with our previous example of 100 kilograms of fish fed at two percent of body weight and producing 58.7 grams of NH_3-N, with 90 percent being oxidized to NO_3-N, it will be necessary to reduce 52.83 grams of NO_3-N per day. On the basis of established rates, the bacteria supported on 227,715 square centimeters of support media can bring about the reduction. This is equivalent to 0.1167 cubic meter of 2.54-centimeter flexirings.

Both laboratory and pilot-level studies have demonstrated the feasibility of continuous denitrification, and it is anticipated that this microbial process for removing nitrogen from fish culture water will gain wider use in the future.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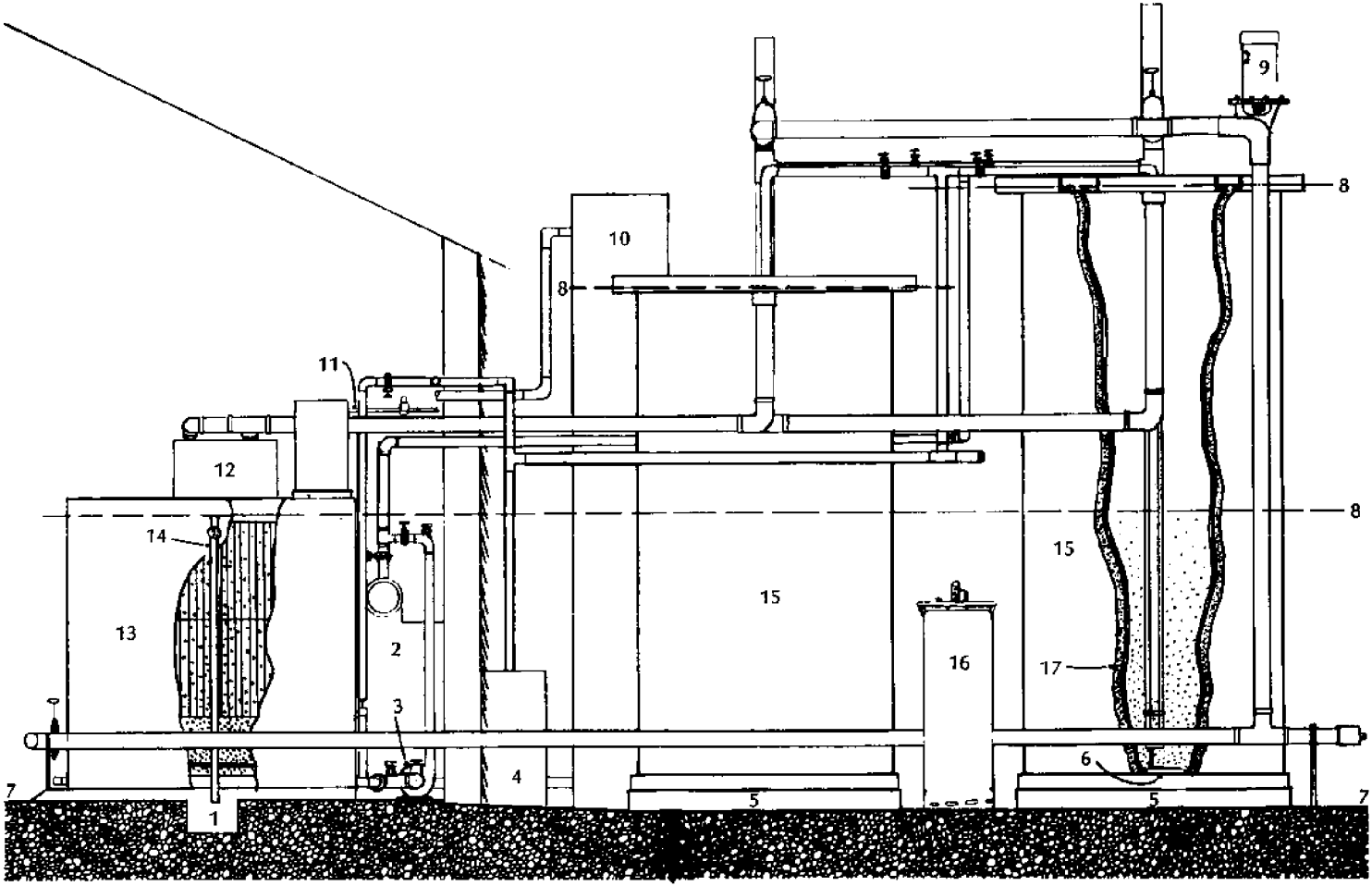

Summary

The efficiency of salmonid production is closely related to the maintenance of optimum culture conditions. Only a limited number of locations throughout the country have adequate supplies of high quality water that meet all the requirements for efficient production. Where water supplies are adequate, temperature is the factor that first limits growth. However, temperature modification in a typical flow-through system is usually prohibitive in cost and is restricted in its application.

To be economical, water re-use systems must provide for efficient water movement. This can be done by proper design and selection of pumps. Lifting water from one part of a system to another should be minimized, and whenever possible gravity flow systems should be given preference over pressurized ones. We have found conveyor pumps to be the most efficient devices available for moving water, and strongly recommend that they be considered as prime movers of water within a culture system.

Heating and cooling requirements are closely related to prevailing ambient temperatures, but good design, use of insulation and minimization of the surface area-to-volume ratio will reduce the requirements to a minimum.

Re-oxygenation of water can be accomplished by a number of methods but we think that direct oxygenation with gaseous oxygen offers many advantages, and we urge its consideration.

When water re-use systems employing secondary and tertiary water treatment are used, some toxic metabolites are present. Water treatment operations should be carried out in a manner that will prevent the build-up of nitrite and hydrogen sulfide. These microbial metabolites are toxic to fish and represent additional critical parameters in a re-use system.

The use of physical filters to remove solid wastes and aeration to restore oxygen does increase the carrying capacity of a given volume of water. Under these conditions water re-use is restricted by the accumulation of toxic metabolic products. The most toxic of the metabolic products is ammonia and most early research has been directed toward either its removal or its conversion to a less toxic compound. The most practical and widely used method for doing the latter is microbiological nitrification. In this process, ammonia is oxidized to nitrite and nitrite is, in turn, oxidized to nitrate.

But in a high-density culture system the nitrate concentration builds up rapidly and can eventually reach toxic levels. The percentage of re-use possible in systems employing this form of secondary water

treatment is limited by the acceptable level of nitrate in the system. Discharging high-nitrate water from the system without further treatment is undesirable. In some installations lagoons are employed where anaerobic conditions enable denitrification to proceed before the water is discharged to the environment. An alternative to this procedure is to employ continuous denitrification or tertiary treatment as an integral part of the water treatment in the culture system. This has been done successfully, and it greatly increases the percentage of water re-use possible and eliminates the need for discharging high-nitrate water or water too high in oxygen demand from the system.

The many advantages of water re-use systems have resulted in their use in both salmonid and catfish production, and indications are that their use will continue to increase.

References

Anon. 1971. Notes on water pollution, no. 52. Water Pollution Research Laboratory, Stevenage, England.

Burrows, R. E. and B. D. Combs, 1968. Controlled environments for salmon propagation. The Progressive Fish-Culturist, 30(3):123-126.

Croker, Morris C., 1973. Personal Communication.

Davies, T. R., 1973. Isolation of bacteria capable of utilizing methane as a hydrogen donor in the process of denitrification. Water Research (Pergamon Press), 7:575-579.

Duddles, G. A. and S. E. Richardson, 1973. Application of plastic media trickling filters for biological nitrification studies. Dow Chemical Company report submitted to U.S. Environmental Protection Agency in fulfillment of Project Number 17010 SUF, Contract Number 14-12-900.

Gigger, R. P., Jr. and R. E. Speece, 1970. Treatment of fish hatchery effluent for recycle. Technical Report No. 67, Engineering Experiment Station, New Mexico State University.

Haug, R. T. and P. L. McCarty, 1971. Nitrification with the submerged filter. Presented at the Annual Conference of the Water Pollution Control Federation, San Francisco, California, October, 1971.

Liao, P. B., 1971. Water requirements of salmonids. The Progressive Fish-Culturist, 33(4):210-215.

Liao, P. B., 1972. Development of hatchery water treatment systems. Presented at the Eleventh Annual Conference Association of Conservation Engineers, New England Center for Continuing Education, Durham, New Hampshire, October 2-5, 1972.

Meade, T. L. and B. R. Kenworthy, 1974. Denitrification in water re-use systems. Presented at the Fifth Annual Workshop, World Mariculture Society, Charleston, South Carolina, January, 1974.

St. Amant, P. P. and P. L. McCarty, 1969. Treatment of high nitrate waters. American Water Works Association Journal, 61(12):659-662.

Schroeder, E. D., 1967. Dissimalatory nitrate reduction by mixed bacterial populations. Doctor of Philosophy Dissertation, Rice University, Houston, Texas.

Smith, J. M., A. M. Masse, W. A. Feigeaud and L. J. Kamphake, 1972. Nitrogen removal from municipal waste water by columnar denitrification. Environmental Science and Technology, 6(3):260-267.

Sousa, R. J., T. L. Meade and R. E. Wolke, 1974. Reduction of ammonia toxicity by salinity and pH manipulation. Presented at the Fifth Annual Workshop, World Mariculture Society, Charleston, South Carolina, January, 1974.

Stankewich, M. J., Jr., 1972. Biological nitrification with the high purity oxygen process. Presented at the Purdue Industrial Waste Conference 27th Annual Meeting, May 2-4, 1972. Purdue University, West Lafayette, Indiana.

Verhoeven, W., 1956. Some remarks on nitrate and nitrite metabolism in microorganisms. *In* A symposium on inorganic nitrogen metabolism: function of metalloflavoproteins. Edited by W. B. McElroy and B. Glass. Baltimore, Maryland: The Johns Hopkins Press.

Westin, D. T., 1973. Nitrate and nitrite toxicity to salmonid fishes in fresh and brackish waters. M.S. Thesis, University of Rhode Island, Kingston, Rhode Island.